IN THE CLAIMS

Please amend claims 1-7, 9, 11-13 and 16-18 as shown on the "Listing of Claims" attached.

LISTING OF CLAIMS

1. (Currently amended) A method for detecting pathogenic mycobacteria in clinical specimens, said method comprising the steps of:

(a) ~~Clearing~~ clearing contaminants from ~~clarifying~~ the clinical specimens, ~~from containment contaminant by conventional methods~~,

(b) treating the processed clinical specimens obtained in step (a) with a modified lysis buffer to inactivate live pathogenic mycobacteria to make the process ~~safe~~ safer for the user,

(c) extracting genomic DNA from the processed clinical specimen obtained from step (b) using a modified method to increase the yield and quality of DNA,

(d) ~~designing Selecting~~ selecting the sequence of SEQ ~~1D No.4~~ ID NO:4 from the DNA obtained in step (c) for specific detection of pathogenic mycobacteria, said ~~designed~~ sequence comprising ~~of~~ selected intergenic region of SEQ ~~1D. No.3~~ ID NO:3, a flanking region containing a portion of the genes mmaA 1 of SEQ ~~1D No. 1~~ ID NO:1 and a portion of the gene mmaA2 of SEQ ~~1D No. 2~~ ID NO:2,

(e) designing and synthesizing a set of specific oligonucleotide primers of SEQ ~~1D No.5~~ ID NO:5, ~~which is~~ the forward primer, and SEQ 1D ~~No.6 ID~~ NO:6, ~~which is~~ the reverse primer, for Polymerase Chain Reaction (PCR) amplification of SEQ ~~1D No.4~~ ID NO:4,

(f) developing a PCR amplification process for specific amplification of SEQ ~~1D No.4~~ ID NO:4 of step (d), and ~~said process comprising using the specific oligonucleotide primers designed and synthesized in step (e) for detecting presence of pathogenic mycobacteria in the clinical specimens, and~~

(g) analyzing the amplified PCR product by restriction fragment length polymorphism (RFLP) analysis for differentiation of the species of the pathogenic mycobacteria~~um. for a quick assessment of HIV co-infection.~~

2. (Currently amended) A method as claimed in claim ~~1~~ 1, wherein the ~~designed~~ selected SEQ ID ~~No.4~~ NO:4 has the following sequence ~~as follows~~:

5'TGGATCCGTTGACCATGAGGTGTAATGCCTTTCCGGACCCTAGGTGGCCTTTCG
GTGC
TTGCACGGAACGCACCGATGCTTCCCCCTCCCCGCATGCTCGAGGCATGCTATCC
GATAC
AGGGCCGCCGCACTAAACCGCGATCGAATTTGCCCAGGTCAGGGAACGGATATGA
GCGGA
CGAGCTACTTGGTCATGGTGAACTGGGCGACGTTGATTAGGCCTCTGCGGAAGCG
CTCCG
CGCATCCGGTCAGATAGTGCATGAAGTTGTTGTAGACCTCTTCGGACTGTACGGC
GATGG
CGCGTTCGCGGGCAGCCTGTAGGTTGGCGGCCCATGCATCGAGAGTCCGTGCGT
AGTGGG
AATTC3'.

3.(Currently amended) A method as claimed in claim ~~1~~ 1, wherein the clinical specimen is selected from the group consisting of sputum, gastric lavage, cerebrospinal fluid, blood, tissue biopsies, bone marrow aspirates and other body fluids or tissues.

4. (Currently amended) A method as claimed in claim ~~1~~ 1, wherein ~~clarification~~ clearing of the specimens in step (a) from the contaminants is carried out by adding to the said specimens a ~~digestion~~ decontamination mix, ~~containing mild alkali, NaOH, tri sodium citrate and a mucolytic agent and guanidinium isothiocyanate in the range of about 0.4 2.5 M~~ followed by concentrating the specimens ~~of~~ by centrifugation~~,~~ .

5. (Currently amended) A method as claimed in claim 4, wherein the ~~digestion~~ decontamination mix ~~containing~~ contains guanidinium isothiocyanate of concentration in the range of ~~about~~ 0.5-2.0 M, mild alkali, ~~NaOH,~~ tri-sodium citrate and a mucolytic agent. ~~and guanidinium isothiocyanate in the range of about 0.5-2.0 M.~~

6. (Currently amended) A method as claimed in claim 1, wherein the DNA in step (c) is extracted from the treated clinical specimen using a modified lysis buffer ~~by inclusion of ingredients comprising guanidinium isothiocyanate in a range of about 0.5-8 M, Tris.Cl pH 7.6 in a range of about 20-100 mM, N-lauryl Sarcosyl in a range of about 0.5-2% w/v,by weight of the buffer, EDTA in a range of about 0.1-20 mM, ß-Mercaptoethanol in a range of about 1-25 mM and NaCl is present in an amount of about 0.2M and purifying the DNA~~ to improve yield and quality of DNA ~~by thorough precipitation by organic solvents~~.

7. (Currently amended) A method as claimed in claim 6, wherein the modified lysis buffer comprises guanidinium isothiocyanate ~~about~~ 4M, Tris.Cl pH 7.6 ~~is about~~ 50 mM, N-lauryl ~~Sacrosyl~~ Sarcosyl ~~is~~ 1% w/v ~~by weight of the buffer~~, EDTA ~~is about~~ 1 mM, ß 2 mercaptoethanol ~~is about~~ 10 mM and NaCl ~~is~~ 0.2M.

8. (Canceled)

9. (Currently amended) A method as claimed in claim 1, wherein the genomic DNA yield is increased ~~in the range of about~~ by 25 to 50%.

10. (Canceled)

11. (Currently amended) A method as claimed in claim 1, wherein high yielding amplification of DNA in step (f) is achieved by the modified touch down PCR cycling conditions, said conditions comprising steps of providing an initial high annealing temperature in the range of ~~about~~ 62-72°C., followed by lowering ~~of~~ the temperature in the range of ~~about~~ 0.1-1.0°C. per PCR cycle for the first 10-25 cycles, then subsequently carrying out 30 PCR cycles at an optimum annealing temperature of ~~about~~ 56-62° C.

12. (Currently amended) A method as claimed in claim 1, wherein high yielding amplification of DNA in step (f) is achieved by modified touch down PCR cycling conditions, said conditions comprising steps of providing an initial high annealing temperature of ~~about~~ 70°C., followed by lowering of the temperature by ~~of about~~ 0.8°C per PCR cycle for the ~~about~~ first 14 cycles, then subsequently lowering the temperature to ~~about~~ 58°C. for another 25 PCR cycles.

13. (Currently Amended) A method as claimed in claim 1, wherein the oligonucleotide primers capable of amplification of intergenic region of SEQ ID ~~No.4~~ NO:4 for detection of pathogenic mycobacteria in clinical specimens are selected from the group consisting of:

a. 5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID ~~No.5~~ NO:5), ~~which is~~ the forward primer, and

b. 5'GGAATTCCACTACGCACGGACTCTC3' (SEQ ID ~~No.6~~ NO:6), ~~which is~~ the reverse primer.

14. (Previously presented) A method as claimed in claim 1, wherein the length of oligomeric primers is between 5 and 100 bases.

15. (Previously presented) A method as claimed in claim 1, wherein the modified lysis buffer provides a cleaner preparation of the DNA.

16. (Currently amended) A method as claimed in claim 1, wherein treatment with the modified lysis buffer in step (b) ~~containing~~ contains 4M guanidinium isothiocyanato that inactivates the live mycobacteria to make the procedure safer ~~fro~~ for the operator.

17. (Currently amended) A diagnostic kit for the detection of pathogenic mycobacteria in clinical specimens, comprising primers selected from the group consisting of:

a. 5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID ~~No.5~~ NO:5), ~~which is~~ the forward

primer, and

b. 5' GGAATTCCACTACGCACGGACTCTC 3' (SEQ ID ~~No.6~~ NO:6), ~~which is~~ the reverse primer.

18. (Currently amended) A method as claimed in claim 1, wherein the contaminant clarified in step (a) comprises mucus and/or live organisms other than mycobacteria.

19. (Currently amended) A set of primers of SEQ ID ~~Nos~~ NOS:5 and 6 comprising:

5' TGGATCCGTTGACCATGAGGTGTAATG 3' (SEQ ID ~~No 5~~ NO:5, forward primer)

5' GGAATTCCACTACGCACGGACTCTC 3' (SEQ ID ~~No. 6~~ NO:6, backward primer)